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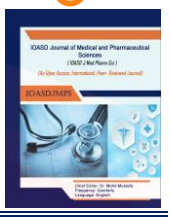

The Status of Widal Test in the Clinical Diagnosis of Typhoid Fever in Imo State, Nigeria

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ABSTRACT

This study examined the usefulness of the Widal test in Imo State, Nigeria, for typhoid disease diagnosis. In the Owerri and Orlu zones of Imo state, 2,700 test subjects-2,300 typhoid fever cases and 400 seemingly healthy individuals—were utilized to examine the incidence of typhoid fever using both the tube and slide agglutination Widal tests. The test findings were contrasted with those from routine blood culture examinations. The test subjects were divided into the following occupational groups: businessmen and women (700), students (400), farmers (450), civil servants (900), and children (250). According to the study's results, the sensitivity, specificity, positive predictive value, and negative predictive value of the Widal tube and slide agglutination tests were 54%, 77%, 90%, and 30%, respectively. A significant difference was found at $p < 0.05$ in the analysis of the data from the two diagnostic tools in the two zones. The Widal test is still useful as a diagnostic tool for typhoid fever in Imo State since it is more practical, affordable, and quick, according to the results.

Keywords: Widal test, clinical diagnosis, typhoid fever, Owerri.

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INTRODUCTION

An essential serological test for the diagnosis of undulant fever and enteric fever is the Widal test. Salmonella enterica serotype typhi is the cause of enteric fever; brucellosis is another name for undulant fever [1]. French physician and bacteriologist Georges Fernand Isidore Widal (1862–1929) established the test in 1896 [2]. Using suspensions of O and H antigens, an agglutination reaction to Salmonella typhi is used to show the presence of lipopolysaccharide (LPS) somatic (O) and flagella (H) agglutinins in a patient's serum in the event of enteric fever [3].

A severe illness with symptoms including fever, headache, abdominal pain, relative bradycardia, splenomegaly, and leukopenia is the hallmark of typhoid fever, a systemic infectious disease [4]. Salmonella enterica serotype typhi is the organism that is its etiologic agent. With an estimated 12 to 33 million cases each year, typhoid fever is a major source of morbidity in many parts of the world [5]. Areas with high rates of urbanization, rapid population expansion, and inadequate access to infrastructure, health care, and drinkable water are more likely to have cases. These consist of Africa, India, and South and Central America. Reports of cases have also come from Eastern Europe [6].

The majority of commercial kits typically contain the antigens for Salmonella paratyphi A, B, and C. The Widal test should be carried out using the tube agglutination technique, which involves testing consecutive two-fold dilutions of the subject's serum ranging from 1:20 to 1:1280 [7]. Later on, a rapid slide test was created, and due to its ease of use, it has become the most widely utilized method in nearby hospitals and laboratories [8]. However, Salmonella typhi must be isolated from bodily fluids such as blood, feces, urine, bone marrow, or other sources in order to provide a conclusive diagnosis of typhoid fever Salmonella typhi isolation and culture facilities are frequently unavailable in developing nations like Nigeria, particularly in smaller hospitals and labs. As a result, diagnosis is based on clinical features and the Widal test's identification of agglutinating antibodies to S. typhi. The Widal test is still the sole useful test accessible in the majority of underdeveloped nations since it has been widely utilized in the serodiagnosis of typhoid fever [10]. Furthermore, the preference for the Widal test has been linked to the

scarcity of microbiological facilities and the lengthy wait times for culture findings [11].

The Widal test has its own limits despite being so widely used due to its simplicity, quickness, and convenience of use. Patients frequently encounter other bacteria in the same genus that cause cross-reactivity, such as Salmonella enteritidis, Salmonella typhimurium, and certain strains of E. coli. Since many people have antibodies against these enteric pathogens, these antibodies also react with the antigens in the Widal test, leading to a false-positive result [12]. Numerous "positive" findings have raised doubts and anxieties, occasionally mistakenly inspiring a dread of endemics.

Consequently, "it is important to carefully interpret test results in light of prior enteric fever episodes, typhoid vaccination history, and the overall level of antibodies in the populations in endemic areas of the world." Another test for typhoid fever diagnosis is the Typhidot test. The absence of sanitary facilities, inappropriate waste disposal, a lack of basic infrastructure, a shortage of potable water, and other issues are some of the issues associated with urbanization in Nigeria, including Imo State.

Predisposing variables for the occurrence and spread of typhoid fever and other public health illnesses include these and other issues. Therefore, it seems sense to assess whether the Widal Test, which is still employed in the majority of our labs and hospitals, should be kept in use as a diagnostic tool for typhoid fever. The information required for the Widal test to remain relevant and reliable in Imo State would be provided by this evaluation.

MATERIALS AND METHODS

The Study Area

The study was conducted in Nigeria's Imo State in the cities of Owerri and Orlu. Nigeria has thirty-six states, including Imo State. It is located in the Imo River Basin, which gives it its name, to the east of the Niger River. Its borders are as follows: Abia State to the east; Rivers State to the south and southwest; the River Niger, which forms the border of Delta State, to the west and northwest; and the States of Anambra and Enugu to the north. Imo state is situated in southeast Nigeria, with latitudes of $5^{o}10^{1}$ north and $5^{o}57^{1}$ north and longitudes of $6^{o}35^{1}$ east and $7^{o}28^{1}$ east, respectively. Its entire land area is approximately 5,067.20 km^. Imo state is made up of three distinct zones namely, Okigwe, Orlu and Owerri. It is also delineated into 27 local government areas.

Like other regions of Nigeria, Imo state experiences two distinct seasons every year: the rainy season, which runs from May to October, and the dry season, which runs from November to April. In August, there is typically a dry spell that is referred to as "August break." Typically, the harmattan period lasts from December to February. Between 1800 and 2500 mm of rain fall on average each year. Relative humidity is typically 74%, and this is usually experienced during the rainy season. The primary forest resources are rubber and palm trees, which are found in the tropical rain forest flora. The population is of Igbo ethnicity. The majority of the population—roughly 95%—are Muslims and traditional believers.

In Imo State, the field of education is flourishing. Banks, hotels, restaurants, and fast-food chains are among the other industries that are growing in number. The majority of the population is made up of students, subsistence farmers, traders, artists, and civil servants.

The study's residents don't have access to consistent water supplies. They turn to wells, streams, borehole water, and commercially produced satchet water, sometimes referred to as "pure water." The lack of routine washing of the overhead tanks in the boreholes and the aging and decaying infrastructure that transports municipal water make it easier for water-borne illnesses like typhoid fever to spread. Food and water pollution from human feces also occurs as a result of low sanitation and hygiene standards.

Test Samples

Blood samples from outpatients and seemingly healthy persons who visited the laboratory sections of The Federal Medical Centre, Owerri, and Imo State University Teaching Hospital (IMSUTH), Orlu, were used in this investigation. For each facility, these samples were taken on average ten (10) times a day from March through September of 2010.

Instrument For Demographic Data Collection

The instrument used to collect demographic and health information data in this study was a structured questionnaire administered on all respondents who voluntarily accepted to participate in the study. The respondents who completed the questionnaires were also the people who volunteered their blood samples for laboratory examination.

Selection of Study Participants

A total of 2,700 participants aged between 7 to 58 years were randomly chosen for this study. This comprises 400 apparently healthy individuals and patients' relatives, and 2,300 out-patients who visited the laboratories of the two hospitals for Widal test examination. The participants were further categorized as civil servants, farmers, students, business men/women, and children. Participants who took antibiotics within 1 month before visiting the hospital were excluded from the study to eliminate false results. The objectives of the study were carefully explained to the participants and their willingness to participate in the study was sought for; those who understood and voluntarily accepted to be included in the study were selected.

Bacteriological Studies

Blood samples by vein puncture were collected from each study participant by a Medical Laboratory Scientist in the laboratory of each of the health institutions. A tunicate was tied at the upper arm of the participant to expose the respondent's vein and avoid stress on the cardiac flow. Alcohol swab was used to clean the site of collection. A sterile disposable 10ml syringe and needle was carefully inserted into the vein and 10ml of blood sample was collected. Five (5) ml of the freshly collected venous blood was inoculated into 25ml of freshly prepared and sterilized glucose broth medium for blood culture. The remaining 5ml of serum was capped and left to stand for 20 minutes for the complete formation of clot and separation of the serum to be used in serological analysis.

Analysis of Samples

Two procedures were used to analyze the blood samples: blood culture was used to isolate Salmonella typhi, the bacterium that causes typhoid fever, and the Widal test was used to diagnose typhoid disease. Prior to conducting the Widal slide agglutination test, each blood sample underwent the Widal tube agglutination test to ascertain the serum/antigen titre level. This would support accurate interpretation of the Slide Agglutination test results, which are primarily utilized in our facilities. Each sample was thereafter subjected to a blood culture in order to confirm the diagnosis of typhoid fever.

(a) The Widal Tube Agglutination test

For each serum sample, the Widal tube agglutination test was done as follows:

Serial Dilutions

With the help of a sterile pipette, a ten-fold serial dilution of the serum was prepared by adding 1ml of serum into 9ml of physiological saline in a test tube. Six (6) test tubes were then prepared in three rows for serial dilutions. The first row is for the *S. typhi* O antigen each containing 0.5ml of the O antigen, the second row for the *S. typhi* H antigen contains 0.5ml each of the H antigen, and the third row for the *S. paratyphi* AH antigen contains 0.5ml each of the AH antigen. The last (6th) tube in each row was used for control where 0.5ml of antigen was added to 0.5ml of physiological saline. A two-fold serial dilution of the initial ten-fold diluted serum was done. This was done by serially transferring 0.5ml of the initial ten-fold diluted serum into each of the 5 test tubes containing the different antigens in the separate rows. This represents 1:20, 1:40, 1:80, 1:160, and 1:320 dilutions for each of the five tubes, respectively. 0.5ml from the 5th tube in each row was discarded. The Widal test tube rack was then incubated overnight at 50^{o}C. The tubes were observed for any clumps/agglutinations (Olopoenia and King, 2000). The smallest quantity of serum that exhibited agglutination was considered the end-point of serum activity or titre and considered significant.

(b) Widal Slide Agglutination test

Each serum sample underwent the slide agglutination test once the titre results were obtained from the tube agglutination test. On a plastic agglutination slide, equal volumes (0.5 ml) of the patient's undiluted serum and the antigens OA, OB, OC, and OD were arranged side by side and manually rocked for a duration of one minute. For the HA, HB, HC, and HD antigens, the identical process was carried out once again. S. paratyphi H antigen is represented by HA, HB, and HC, whereas S. paratyphi O antigen is represented by OA, OB, and OC. The S. typhi H and O antigens are denoted by HD and OD, respectively. Positive or negative agglutinations were observed. Agglutinations were considered statistically significant and recorded as positive when they were greater than or equal to the titre indicated by the tube agglutination test.

(c) Blood Culture

For each blood sample collected, 5ml was inoculated into 25ml of 10% glucose broth medium and incubated overnight at 37^{o}C. The next day, two subcultures from this broth culture were done on two MacConkey agar Plates and incubated at 37^{o}C for 24hours. The MacConkey agar plates were later examined for the cultural characteristics of *Salmonella typhi* (moist, circular, smooth convex and pale yellow colonies 2-3mm in diameter). Blood samples were noted as positive or negative, subject to confirmation using biochemical tests.

Biochemical Tests

To verify the existence of Salmonella, the blood culture isolates of every sample underwent the following biochemical tests: lactose fermentation, glucose fermentation, citrate utilization, motility test, and Gram reaction.

Demographic and Health Information Studies
Administration of Questionnaire

A structured questionnaire consisting of two sections: (a) Demographic section and (b) Health information, was administered on all participants. The demographic section was to elicit necessary demographic information on the respondents, while the health information section was intended to evaluate the level of awareness of the respondent on typhoid fever disease.

The questionnaire was validated or pre-tested before administering it on the participants. This validation was done by pre-testing the questionnaire on 10 respondents attending the out-patient section of The Umezuruike Hospital, Owerri. Difficulties experienced while answering the questions were noted and taken into consideration before the final questionnaire was prepared and used for study. Irrelevant questions were removed and important suggestions were added. The objectives of the study were lucidly explained to the participants, assuring them of the confidentiality of their information.

Field assistants assisted those who were not literate enough to complete the questionnaire on their own. All questionnaires were completed and collected on the spot.

Ethical Consideration

The Institutional Review Board of the Imo State University, Owerri reviewed and approved this study. Informed consent was obtained from all study participants according to the guidelines of the Institutional Review Board of Imo State University and as stipulated by the World Health Organisation.

Data Analysis

The data obtained from the tests were subjected to statistical analysis using Microsoft Office Excel package 2007 version. Tables, charts, and analysis of variance (ANOVA) were used to interpret the data.

RESULTS

Number of Patients Examined

A total number of two thousand seven hundred (2,700) individuals were examined. Of this number, 1,483 (55%) were from Owerri zone while 1,217 (45%) were from Orlu zone. Four hundred (400) apparently healthy individuals and 2,300 patients were altogether examined. These numbers are further broken down according to occupations into Civil servants (900), Farmers (450), Students (400), Businessmen (700), and Children (250) (Table 1).

Table 1: Number of individuals examined in the two zones across occupations

	Owerri			Orlu			
	Healthy	**Patients**	**Total**	**Healthy**	**Patients**	**Total**	**Totals**
Civil Servants	58	545	603	42	255	297	900
Farmers	35	150	185	65	200	265	450
Students	28	247	275	22	103	125	400
Businessmen	42	248	290	58	352	410	700
Children	30	100	130	20	100	120	250
Grand Totals	193	1290	1483	207	1010	1217	2700

Results of Widal Tube Agglutination Test

The results of the Widal tube agglutination test are presented in Table 2. Titre values from 1:80 and above were regarded as significant and therefore positive for the *Salmonella* antigen.

Table 2: Positive Titre Values (≥1:80) for the Widal Tube Agglutination Test.

	Owerri zone						**Orlu Zone**					
Titre Values	1:80		1:160		1:320		1:80		1:160		1:320	
	Healthy	Patients	Healthy	Patients	Healthy	Patients	Healthy	Patients	Healthy	Patients	Healthy	Patients
Civ. Servants	8	105	8	216	12	90	5	32	6	70	9	130
Farmers	5	12	7	33	13	40	3	46	7	64	10	46
Students	0	10	2	79	3	91	0	0	1	20	4	61
Businessmen	1	34	3	65	7	55	2	30	6	70	7	180
Children	3	20	7	18	10	22	1	22	3	24	6	36

Results of Widal Slide Agglutination Test

A total of 1872 (69%) out of the 2700 individuals had significant (O Titre ≥ 1:80, H titre ≥ 1:80) slide agglutination titre values and therefore were regarded as positive. This number includes 159 (40%) of all 400 healthy individuals and 1,713 (75%) of all 2,300 sick individuals examined in the two zones (Table 3).

Table 3: Widal Slide Agglutination Test Results for the two zones

	Owerri zone				**Orlu zone**			
	Healthy		**Patients**		**Healthy**		**Patients**	
	Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
Civil Servants	28	30	411	134	20	22	232	23
Farmers	25	10	85	65	20	45	156	44
Students	5	23	180	67	5	17	81	22
Businessmen	11	31	154	94	15	43	280	72
Children	20	10	52	48	10	10	82	18
Totals	89	104	882	408	70	137	831	179

Owerri Zone

In Owerri zone 1,483 individuals were examined. Nine hundred and seventy-one (971) (65%) out of this number were positive for significant titre values. This includes 89 (43%) out of 193 healthy persons and 882 (68%) of 1290 sick patients (Table 4).

Table 4: Results of Widal Slide Agglutination tests for Owerri Zone

	Healthy		Patients		Totals
	Positive	Negative	Positive	Negative	
Civil Servants	20	22	232	23	297
Farmers	20	45	156	44	265
Students	5	17	81	22	125
Businessmen	15	43	280	72	410
Children	10	10	82	18	120
Totals	70	137	831	179	1217

Orlu zone

There were 1,217 individuals examined. Nine hundred and one (901) (74%) individuals were positive for typhoid fever. This represents 70 (34%) of 207 healthy individuals and 831 (82%) of 1,010 sick patients (Table 5).

Table 5: Results of Widal Slide Agglutination tests for Orlu Zone

	Healthy		Patients		Totals
	Positive	Negative	Positive	Negative	
Civil Servants	28	30	411	134	603
Farmers	25	10	85	65	185
Students	5	23	180	67	275
Businessmen	11	31	154	94	290
Children	20	10	52	48	130
Totals	89	104	882	408	1483

Blood Culture Test Results

Blood or bone marrow culture is widely accepted as the definitive tool for the diagnosis of typhoid fever. Isolation of the parasite (*Salmonella typhi*) in the blood indicates bacteremia. This test represents the "Gold Standard" for the comparison of other test results for *Salmonella typhi* infection. In this study, 973 (36%) of the 2,700 individuals tested positive for *Salmonella typhi* infection as shown by growth on MacConkey agar. This represents 53 (13%) of 400 healthy individuals and 920 (40%) of 2300 sick patients (Table 6). This number (973) falls short of the 1,872 "positive" results from the slide agglutination test, indicating overdiagnosis.

Table 6: Blood culture test results for the two zones

	Owerri zone				Orlu zone			
	Healthy		Patients		Healthy		Patients	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Civil Servants	11	47	325	220	8	34	70	185
Farmers	4	31	48	102	5	60	76	124
Students	1	27	58	189	3	19	46	57
Businessmen	4	38	42	206	8	50	180	172
Children	3	27	23	77	6	14	52	48
Totals	23	170	496	794	30	177	424	586

Owerri zone

A total of 1,483 individuals were examined in Owerri zone. Five hundred and nineteen (519) (35%) tested positive for *Salmonella typhi*. This represents 23 (12%) of 193 healthy individuals and 496 (38%) of 1,290 sick patients (Table 7). The number of these true positive diagnoses is less than that derived from the slide agglutination test, indicating overdiagnosis.

Table 7: Blood Culture test results for Owerri zone

	Healthy		Patients		Totals
	Positive	Negative	Positive	Negative	
Civil Servants	11	47	325	220	603
Farmers	4	31	48	102	185

Students	1	27	58	189	275
Businessmen	4	38	42	206	290
Children	3	27	23	77	130
Totals	23	170	496	794	1483

Orlu zone

Out of the 1,217 individuals tested in Orlu zone, 454 (37%) were positive for *Salmonella typhi* infection while 763 (63%) were negative. Of the positive results, 30 (14% of 207) were healthy individuals while 424 (42% of 1,010) were sick patients (Table 8).

Table 8: Blood Culture Test Results for Orlu Zone

	Healthy		Patients		Totals
	Positive	**Negative**	**Positive**	**Negative**	
Civil Servants	8	34	70	185	297
Farmers	5	60	76	124	265
Students	3	19	46	57	125
Businessmen	8	50	180	172	410
Children	6	14	52	48	120
Totals	30	177	424	586	1217

Results of Biochemical Tests

Biochemical tests carried out on the blood culture isolates of each sample were Gram staining, motility test, lactose fermentation test, glucose fermentation test, and citrate utilization test, for the two zones. The results are presented in Table 9.

Table 9: Results of Biochemical tests

Groups	No. of Isolates Examined	Gram Staining	Motility	Lactose Ferm.	Glucose Ferm.	Citrate Utilization
Civil Servants	414	-	+	-	+	-
Farmers	133	-	+	-	+	-
Students	108	-	+	-	+	-
Businessmen	234	-	+	-	+	-
Children	84	-	+	-	+	-

Accuracy of the Widal Slide Agglutination Test

It is important to compare the results of the Widal slide agglutination test in this study with those of the standard – Blood culture. This will enable us to calculate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the slide agglutination test.

Overall Accuracy of the Slide Agglutination Test

We first calculate the True Positives, True Negatives, False Positives, and False Negatives for the entire two zones.

True Positives = Number of patients correctly diagnosed as sick

= (496 from Owerri zone + 424 from Orlu zone)

= 920

True Negatives = Number of healthy persons correctly identified as healthy

= (170 from Owerri zone + 177 from Orlu zone)

= 347

False Positives = Number of healthy persons incorrectly diagnosed as sick

= [Number of "positive" healthy persons in the two regions from Slide Agglutination test results] minus [Number of positive healthy persons from blood culture results].

= (89-23) + (70-30)

= 106

False Negatives = Number of sick patients incorrectly identified as healthy

= [Number of "negative" sick patients in the two regions from blood culture results] minus [Number of "negative" sick patients from Slide Agglutination test]

= (794 - 408) + (586 – 179)

= 793

$$\text{Sensitivity} = \frac{TP}{TP + FN} = \frac{920}{920 + 793} \times 100\% = 54\%$$

$$\text{Specificity} = \frac{TN}{TN + FP} = \frac{347}{347 + 106} \times 100\% = 77\%$$

Positive Predictive Value (PPV) = $\frac{TP}{TP + FP}$ X 100% = 90%

Negative Predictive Value (NPV) = $\frac{TN}{TN + FN}$ X 100% = 30%

Accuracy of the Slide Agglutination Test in the Two Zones

Using the foregoing formulae, the Sensitivity, Specificity, Positive Predictive value, and Negative Predictive value of the Slide agglutination test for the Owerri and Orlu zones were calculated and are presented in Table 10. While the sensitivity of the test was greater in Owerri (56%) than in Orlu (51%), the specificity was greater in Orlu (82%) than in Owerri (72%). The positive predictive value was greater in Orlu (91%) than in Owerri (88%), while the negative predictive value was greater in Owerri (31%) than in Orlu (30%).

Table 10: Overall and Zonal accuracy of the Widal Slide Agglutination test

	Owerri	Orlu	Overall
True Positive	496	424	920
True Negative	170	177	347
False Positive	66	40	106
False Negative	386	407	793
Sensitivity (%)	56	51	54
Specificity (%)	72	82	77
Positive Predictive Value (%)	88	91	90
Negative Predictive Value (%)	31	30	30

Comparison Between Standard and Test Results in the Two Zones

Table 11 shows the positive results gotten from Slide Agglutination test and those from the Standard test (Blood Culture). The differences between these values were statistically compared using Analysis of Variance (ANOVA) at 0.05 level of significance.

Table 11: Comparison between Standard and Test Results

	Owerri				Orlu			
	Standard		Slide		Standard		Slide	
	Healthy	Patients	Healthy	Patients	Healthy	Patients	Healthy	Patients
Civil Servants	11	325	28	411	8	70	20	232
Farmers	4	48	25	85	5	76	20	156
Students	1	58	5	180	3	46	5	81
Businessmen	4	42	11	154	8	180	15	280
Children	3	23	20	52	6	52	10	82

General Pre-Test Health Information of Respondents

This section describes the general health information of all respondents in the two zones visited; number of those who were sick and number of individuals healthy.

There were 100 healthy civil servants and 800 sick civil servants; 100 healthy farmers and 350 sick farmers; 50 healthy students and 350 sick students; 100 businessmen/women and 600 sick businessmen/women; and 50 healthy chidren against 200 sick children.

General Post-Test Health Information of Respondents

After blood culture tests, 19 healthy civil servants and 395 sick civil servants tested positive for blood culture results. Also positive for blood culture tests were 9 healthy farmers and 124 sick farmers; 4 healthy students and 104 sick students; 12 healthy businessmen and 222 sick businessmen; and 9 healthy children and 75 sick children.

Duration of Illness for Sick Respondents

Question 7 of the questionnaire asked the sick respondents how long they have been sick before reporting to the hospital. Most of the respondents are visiting the hospital during the first week of onset of illness. Below are the respondents' answers across the occupations for the two zones presented in Table 12.

Table 12: Answers to duration of illness by sick Respondents

	≤ 1 week (%)	8days - 2 weeks (%)	> 2weeks (%)
Civil servants	640 (80)	120 (15)	40 (5)
Farmers	140 (40)	175 (50)	35 (10)
Students	266 (76)	67 (19)	18 (5)
Businessmen	312 (52)	168 (28)	120 (20)
Children	180 (90)	20 (10)	0 (0)

Level of Awareness of Respondents to Typhoid Fever

Questions 11 and 12 of the questionnaire sought to elicit information on the level of awareness of respondents to the causes and prevention of typhoid fever. Ninety (90%) percent of civil servants, 60% of farmers, 85% of students, 56% of Businessmen/women, and 51% of children all showed adequate knowledge of the causes and prevention of typhoid fever. Their general level of awareness is presented in Table 13.

Table 13 Level of Typhoid fever awareness among respondents

	Total Number	Aware (%)	Not aware (%)
Civil servants	900	810 (90)	90 (10)
Farmers	450	270 (60)	180 (40)
Students	400	340 (85)	60 (15)
Businessmen	700	392 (56)	308 (44)
Children	250	128 (51)	122 (49)

DISCUSSION

In underdeveloped nations, the most widely used, accessible, and common diagnostic test for typhoid fever is the Widal test. It uses suspensions of O and H antigens to show that the patient's serum contains somatic (O) and flagellar (H) agglutinins to Salmonella typhi. A percentage of people in any given location have serum that has antibodies that can respond to a varied titer in the Widal test. The frequency of 'H' agglutinins in a population, in the absence of prior typhoid or TAB vaccine inoculation, reflects that population's experience of Salmonellae with the corresponding antigens- either as enteric fever or as a latent infection-and thus differs greatly between nations and regions [13]. In contrast, the frequency and concentration of 'O' agglutinins exhibit significantly less variation across different regions of the globe. Data from a number of studies have raised serious questions about the usefulness of the Widal test in typhoid illness diagnosis. This ambiguity has been influenced by a number of things. These consist of inadequately standardized antigens, the exchange of antigenic determinants with other Salmonellae, the consequences of antibiotic therapy, and prior administration of the TAB vaccination. The challenge of interpreting Widal test results in S. typhi-endemic locations, where the titres of antibodies in the normal population are frequently unknown, is another significant issue.

However, the definitive test for the clinical diagnosis of typhoid fever is either a 4-fold increase in titre of sera taken 10-14 days apart, or a blood or bone marrow culture. Recent scientific advances have also introduced the dip-stick assay for *Salmonella typhi*-specific IgM antibodies [15].

The goal of this study was to evaluate the validity of the Widal test, also known as slide agglutination, which is utilized in the majority of Nigerian laboratories [16], including those in Imo State. But before doing the slide agglutination test, the titre levels were ascertained by the tube agglutination test. The most used test is the slide agglutination Widal test due to its affordability, simplicity, and quickness [17]. The evaluation of this test can be done by contrasting it with a regular bone marrow or blood culture.

The majority of the study participants visited the hospital during the first week of becoming ill. Using the slide agglutination Widal test (for titres ≥ 1:160), 1,872 (69%) of the 2,700 individuals investigated in the Owerri and Orlu zones tested positive in this study, compared to 973 (36%) who used blood culture. Out of the 1,483 people in the Owerri zone, 971 (65%) tested positive for Widal, while 519 (35%), tested positive for blood culture. Of the 1,217 people in the Orlu zone, 901 (74%) were Widal positive and 454 (37%) were culture positive. Though there were more confirmed cases in this work than in the latter, the results are consistent with those of a comparable study conducted in Ebonyi state by [17], in Borno and Plateau states by [18], and in Kano by [19]. Blood culture is still the recommended diagnostic procedure for typhoid fever because numerous studies have demonstrated that the Widal test is not very useful in typhoid endemic areas [20].

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There were more cases of diagnoses from both the Slide agglutination test and blood culture tests in Orlu zone than in Owerri zone. This might be attributable to the poor sanitary environment and inadequate sources of potable water in Orlu zone when compared with that of Owerri. It has been suggested that poor sanitary environment and the intake of unsafe water are predisposing factors to the incidence of typhoid fever infection [22]. Nonetheless, the studied population in the two locations had a high degree of awareness of the causes and prevention of typhoid fever.

The results of this study's Slide Agglutination Widal test showed high values for sensitivity, specificity, positive predictive value, and negative predictive value in both zones. The results of [23, 24]'s investigation on the "value of a single Widal test in the diagnosis of typhoid fever" are comparable to these values.

An analysis of variance (ANOVA) comparing the positive Widal test findings with the blood culture results at $p<0.05$ revealed substantial discrepancies in the results, suggesting that the population means are not comparable.

CONCLUSION

The Widal Slide agglutination test's continuing application and relevance will be evaluated by comparing the findings to the conventional blood culture results in this study. It is recommended to use O and H agglutinin titers of $\geq 1/80$, as used in this inquiry, since they are believed to be crucial for diagnosis. When blood cultures cannot be acquired, the Widal slide agglutination test is a simple, affordable, and generally noninvasive diagnostic technique that can be useful. Typhoid fever can still occur, even in the event that the results are negative, so it's important to carefully go over the data. One might conclude that the test is still useful and relevant for clinically diagnosing typhoid fever based on the study's outstanding accuracy. The positive, specificity, and sensitivity of the test. Notably high were the test's positive predictive value, negative predictive value, sensitivity, and specificity.

REFERENCES

1. MAHLE, W. T., & LEVINE, M. M. (1993). Salmonella typhi infection in children younger than five years of age. *The Pediatric infectious disease journal*, *12*(8), 627-631.
2. Bhan, M. K., Bahl, R., Sazawal, S., Sinha, A., Kumar, R., Mahalanabis, D., & Clemens, J. D. (2002). Association between Helicobacter pylori infection and increased risk of typhoid fever. *The Journal of infectious diseases*, *186*(12), 1857-1860.
3. Choo, K. E., Razif, A. R., Oppenheimer, S. J., Ariffin, W. A., Lau, J., & Abraham, T. (1993). Usefulness of the Widal test in diagnosing childhood typhoid fever in endemic areas. *Journal of paediatrics and child health*, *29*(1), 36-39.
4. Luxemburger, C., Duc, C. M., Lanh, M. N., Wain, J., Hien, T. T., Simpson, J. A., ... & Farrar, J. J. (2001). Risk factors for typhoid fever in the Mekong delta, southern Viet Nam: a case-control study. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *95*(1), 19-23.
5. Massi, M. N., Gotoh, A., Bishnu, A., Kawabata, M., Shirakawa, T., & Hatta, M. (2003). Rapid diagnosis of typhoid fever by PCR assay using one pair of primers from flagellin gene of Salmonella typhi. *Journal of infection and chemotherapy*, *9*(3), 233-237.
6. Mastroeni, P., & Menager, N. (2003). Development of acquired immunity to Salmonella. *Journal of medical microbiology*, *52*(6), 453-459.
7. Crump, J. A., Barrett, T. J., Nelson, J. T., & Angulo, F. J. (2003). Reevaluating fluoroquinolone breakpoints for Salmonella enterica serotype Typhi and for non-Typhi salmonellae. *Clinical Infectious Diseases*, *37*(1), 75-81.
8. Cunha, B. A. (2004). Osler on typhoid fever: differentiating typhoid from typhus and malaria. *Infectious Disease Clinics*, *18*(1), 111-125.
9. Levine, M. M., Tacket, C. O., & Sztein, M. B. (2001). Host–Salmonella interaction: human trials. *Microbes and infection*, *3*(14-15), 1271-1279.
10. Everest, P., Wain, J., Roberts, M., Rook, G., & Dougan, G. (2001). The molecular mechanisms of severe typhoid fever. *Trends in microbiology*, *9*(7), 316-320.
11. Lakshmi, V., Ashok, R., Susmita, J., & Shailaja, V. V. (2006). Changing trends in the antibiograms of Salmonella isolates at a tertiary care hospital in Hyderabad. *Indian journal of medical microbiology*, *24*(1), 45-48.
12. Patel, J. C., Rossanese, O. W., & Galan, J. E. (2005). The functional interface between Salmonella and its host cell: opportunities for therapeutic intervention. *Trends in pharmacological sciences*, *26*(11), 564-570.
13. Pearson, R. D., & Guerrant, R. L. (2000). Enteric fever and other causes of abdominal symptoms with fever. *Principles and practice of infectious diseases, 5th ed. Churchill Livingstone, New York, NY*, 1136-1150.
14. Schoenen, D. (2002). Role of disinfection in suppressing the spread of pathogens with drinking water: possibilities and limitations. *Water research*, *36*(15), 3874-3888.
15. Lim, M. L., & Wallace, M. R. (2004). Infectious diarrhea in history. *Infectious Disease Clinics*, *18*(2), 261-274.
16. Ashbolt, N. J. (2004). Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology*, *198*(1-3), 229-238.

17. Renuka, K., Kapil, A., Kabra, S. K., Wig, N., Das, B. K., Prasad, V. V. S. P., ... & Seth, P. (2004). Reduced susceptibility to ciprofloxacin and gyra gene mutation in North Indian strains of Salmonella enterica serotype Typhi and serotype Paratyphi A. *Microbial drug resistance*, *10*(2), 146-153.
18. Crump, J. A., Luby, S. P., & Mintz, E. D. (2004). The global burden of typhoid fever. *Bulletin of the World Health Organization*, *82*(5), 346-353.
19. Frimpong, E. H., Feglo, P., Essel-Ahun, M., & Addy, P. A. (2000). Determination of diagnostic Widal titres in Kumasi, Ghana. *West African Journal of Medicine*, *19*(1), 34-38.
20. Garmory, H. S., Brown, K. A., & Titball, R. W. (2002). Salmonella vaccines for use in humans: present and future perspectives. *FEMS microbiology reviews*, *26*(4), 339-353.
21. Slinger, R., Desjardins, M., McCarthy, A. E., Ramotar, K., Jessamine, P., Guibord, C., & Toye, B. (2004). Suboptimal clinical response to ciprofloxacin in patients with enteric fever due to Salmonella spp. with reduced fluoroquinolone susceptibility: a case series. *BMC Infectious Diseases*, *4*, 1-4.
22. Hoffman, S. L., Flanigan, T. P., Klaucke, D., Leksana, B., Rockhill, R. C., Punjabi, N. H., ... & Moechtar, M. A. (1986). The Widal slide agglutination test, a valuable rapid diagnostic test in typhoid fever patients at the Infectious Diseases Hospital of Jakarta. *American journal of epidemiology*, *123*(5), 869-875.
23. Ochiai, R. L., Wang, X., Von Seidlein, L., Yang, J., Bhutta, Z. A., Bhattacharya, S. K., ... & Clemens, J. D. (2005). Salmonella paratyphi A rates, Asia. *Emerging infectious diseases*, *11*(11), 1764.
24. Gabriella, S. (2000). A review of the Salmonellosis surveillance systems in Italy: evolution during the course of time within the international framework. *European journal of epidemiology*, *16*, 861-868.